

WHAT IS CLAIMED

1. A neo-cartilage construct for *in situ* implanting into a cartilage lesion, said construct comprising a cultured  
5 differentiated autologous or heterologous chondrocytes or cells which could be differentiated into chondrocytes incorporated into a support matrix and subjected to an algorithm of the invention wherein said algorithm comprises variable hydrostatic or atmospheric pressure or non-pressure conditions, variable  
10 rate of perfusion, variable medium composition, variable temperature, variable concentration of oxygen or carbon dioxide, variable cell density and variable time to which the chondrocytes are subjected.

15 2. The construct of claim 1 wherein the support matrix is a sponge, porous scaffold or a hydrogel prepared from a material selected from the group consisting of a Type I collagen, a Type II collagen, a Type IV collagen, a cell-contracted collagen containing proteoglycan, a cell-contracted collagen containing  
20 glycosaminoglycan, a cell-contracted collagen containing a glycoprotein, gelatin, agarose, hyaluronin, fibronectin, laminin, a bioactive peptide growth factor, cytokine, elastin, fibrin, a synthetic polymeric fiber made of a polylactic acid, a synthetic polymeric fiber made of a polyglycolic acid, a  
25 synthetic polymeric fiber made of a polyamino acid, polycaprolactone, a polyamino acid, a polypeptide gel, a polymeric thermo-reversible gelation hydrogel (TRGH), a copolymer thereof and a combination thereof.

30 3. The construct of claim 2 wherein the support matrix is the TRGH.

4. The construct of claim 3 wherein the hydrostatic pressure is a cyclic or constant pressure.

5. The construct of claim 4 wherein the hydrostatic pressure is from about zero MPa to about 10 MPa above atmospheric pressure at about 0.01 to about 1 Hz, wherein the time for applying the hydrostatic pressure is from zero to about  
5 24 hours per day for from about one day to about ninety days, wherein said hydrostatic pressure is preceded or followed by a period of zero to about 24 hours per day of a static atmospheric pressure for from about one day to about ninety days, wherein the flow rate is from about 1  $\mu\text{L}/\text{min}$  to about 500  $\mu\text{L}/\text{min}$ ,  
10 wherein the cell density is from about 3 to 60 millions and wherein the oxygen concentration is from about 1 to about 20%.

6. The construct of claim 5 wherein the hydrostatic cyclic pressure is from about 0.05 MPa to about 3 MPa at 0.1 to about  
15 0.5 Hz or constant pressure is from about zero to about 3 MPa above atmospheric pressure and wherein such pressure is applied for about 7 to about 28 days.

7. The construct of claim 6 wherein said hydrostatic  
20 pressure is preceded or followed by a period of about zero to about 28 days of atmospheric pressure.

8. The construct of claim 7 wherein said chondrocytes are autologous.

25

9. The construct of claim 7 wherein said chondrocytes are heterologous.

10. A method for fabrication of a three-dimensional neo-cartilage construct for *in situ* implantation into a cartilage lesion, said method comprising steps:

- a) preparing a support matrix structure;
- 5 b) harvesting a piece of cartilage from a donor for isolation of chondrocytes;
- c) culturing and expanding the chondrocytes;
- d) suspending the expanded chondrocytes in a suspension fluid;
- 10 e) incorporating said suspended chondrocytes into said matrix; and
- f) propagating said chondrocytes into two or three-dimensional neo-cartilage construct using an algorithm of the invention.

15

11. The method of claim 10 wherein the support matrix structure is prepared from a material selected from the group consisting of collagen, a Type I collagen, a Type II collagen, a Type IV collagen, a cell-contracted collagen containing a  
20 proteoglycan, a cell-contracted collagen containing a glycosaminoglycan, a cell-contracted collagen containing a glycoprotein, gelatin, agarose, hyaluronin, fibronectin, laminin, a bioactive peptide growth factor, a cytokine, elastin, fibrin, a synthetic polymeric fiber made of a polylactic acid,  
25 a synthetic polymeric fiber made of a polyglycolic acid, a synthetic polymeric fiber made of a polyamino acid, polycaprolactone, a polyamino acid, a polypeptide gel, a hydrogel, a copolymer thereof and a combination thereof.

30 12. The method of claim 11 wherein the support matrix is prepared from collagen, the Type I collagen, the Type II collagen or the Type IV collagen.

13. The method of claim 12 wherein said collagen, the Type I collagen, the Type II collagen or the Type IV collagen is freeze-dried or lyophilized into a sponge or a sponge-like structure.

5

14. The method of claim 11 wherein the support matrix is the polymeric thermo-reversible gelling hydrogel (TRGH) or a polymeric sol-gel hydrogel.

10 15. The method of claim 11 wherein said cultured and expanded chondrocytes are incorporated into said support matrix suspended in a gel solution.

15 16. The method of claim 15 wherein the support matrix is the collagen sponge or a sponge-like structure and wherein the chondrocytes are incorporated into said sponge in the TRGH or sol-gel hydrogel.

20 17. The method of claim 16 wherein the chondrocytes are incorporated into said sponge at a density of from about 3 to about 60 millions cells/ml.

25 18. The method of claim 17 wherein the hydrostatic pressure is from about zero MPa to about 10 MPa above atmospheric pressure at about 0.01 to about 1 Hz, wherein the time for applying the hydrostatic pressure is from zero to about 24 hours per day for from about one day to about ninety days, wherein said hydrostatic pressure is preceded or followed by a period of zero to about 24 hours per day of a static atmospheric  
30 pressure for from about one day to about ninety days, wherein the flow rate is from about 1  $\mu\text{L}/\text{min}$  to about 500  $\mu\text{L}/\text{min}$ , wherein the cell density is from about 12 to 15 millions and wherein the oxygen concentration is from about 1% to about 20%.

19. The method of claim 18 wherein the hydrostatic cyclic pressure is from about 0.05 MPa to about 3 MPa at 0.1 to about 0.5 Hz or constant pressure is from about zero to about 3 MPa above atmospheric pressure and wherein such pressure is applied  
5 for about 7 to about 28 days.

20. The method of claim 19 wherein said hydrostatic pressure is preceded or followed by a period of about zero to about 28 days of atmospheric pressure.

10

15

20

25

30